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Poor implementation of non-invasive sampling in wildlife genetics studies

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Abstract

The Earth's biodiversity is currently experiencing immense pressure from habitat loss, overexploitation, global climate change, and invasive species, which escalate the global extinction crisis. Comprehensive knowledge of the extent and impact of biodiversity loss is therefore critical for determining species vulnerability and prioritizing conservation goals. An integral part of wildlife conservation research and management is nowadays genetic sampling. Animal DNA has been traditionally obtained invasively, from blood or other tissues, however public concerns over animal welfare require that animals are affected as little as possible during research. One of the ways to minimize the impact on wildlife animal welfare is to use non-invasive genetic sampling. Even though non-invasive genetic sampling techniques have been developed for many animal species, it is not clear how often they are being implemented. Here, I present an overview of recently published articles on genetics in amphibians, birds, carnivores, molluscs and rodents, for which I examined whether they used a lethal, invasive or non-invasive DNA sampling technique. Disappointingly, only 22% of the identified relevant studies implemented the available non-invasive genetic sampling method. I conclude highlighting the need for better implementation of non-invasive DNA collection methods in wildlife research through raising awareness, increasing financial support, and introducing more stringent criteria for obtaining research permits.

Keywords

Non-invasive methods, genetic sampling, DNA, wildlife conservation, animal welfare

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Introduction

The current pace of habitat destruction, introduction of invasive species, overexploitation and climate change has resulted in a rapid loss of biodiversity (May 2010; Bellard et al. 2012; Pimm et al. 2014). This loss has been so large that some scientists talk about the 6th mass extinction (Barnosky et al. 2011; Ceballos et al. 2015). Nowadays, approximately one in five vertebrate species is categorized as vulnerable, endangered or critically endangered by the IUCN's Red List (IUCN 2018). Furthermore, the World Wildlife Fund reported that the average abundance of four thousand species that were monitored during the years 1970–2014 declined by 60% (WWF 2018). Such declines in animal populations often cascade into ecosystem functioning and provisioning services, thus affecting also human well-being (Dirzo et al. 2014). In order to be able to design effective conservation measures and priorities, it is crucial to monitor and assess the impact of biodiversity loss through scientific research (Hoffmann et al. 2010; Carroll et al. 2018).

Scientists can however face several challenges, including public opposition, if the study subject is an animal. The public has long been concerned about the welfare of experimental animals, which is documented by the adoption of legal restrictions on animal use (e.g. the EU Directive 2010/63/EU on the protection of animals used for scientific purposes). Polls and surveys revealed that society in general requires that the pain and distress inflicted on research animals is minimized (Lund et al. 2014; European Commission 2016). While researchers in medical and toxicological fields may enjoy some leniency from the public under the premise that their experiments on animals could save human lives, researchers in ecology and wildlife biology might not get the same privilege (McMahon et al. 2012).

Since declining animal populations are particularly vulnerable to the consequences of decreased genetic variability and inbreeding depression, many wildlife studies include genetic assessment. Genetic tools are particularly suitable for estimating several demographic parameters that reflect responses to changes in the landscape and exploitation (Stetz et al. 2011). Traditionally, DNA samples have been obtained invasively, from blood or other tissues, which sometimes involves euthanization of the animal. Recently, wildlife genetics studies have been however revolutionized by innovative non-invasive sampling techniques. Non-invasively obtained genetic samples allow for data collection without the need for killing or sometimes even handling the animal (Waits and Paetkau 2005). As a source of DNA can be used faeces, hair, urine, saliva, feathers, eggshells, or water in which animals reside (Beja-Pereira et al. 2009; Table 1). Non-invasive genetic sampling was implemented for the first time in wild animals more than twenty years ago (Hoss et al. 1992; Morin et al. 1993). Since then it has been used in a large number of studies that evaluated species abundance (e.g. Piaggio et al. 2016), genetic diversity (Epps et al. 2005), gene flow (Kormann et al. 2012; Karssene et al. 2018), population size (Solberg et al. 2006; Zarzoso-Lacoste et al. 2018), phylogeography (Morin et al. 1993) or hybridization (Anile et al. 2014; Velli et al. 2015).

While non-invasive genetic sampling may yield low quality or quantity of DNA resulting in the occurrence of genotyping errors (Smith and Wang 2014), there are now many protocols available that maximise amplification success and minimize error

rates (e.g. Taberlet et al. 1999; Waits and Paetkau 2005; Beja-Pereira et al. 2009; Wang 2016). Furthermore, it has been shown that non-invasive sampling techniques can be even more cost-effective than traditional methods (Solberg et al. 2006; De Barba et al. 2010), and they often allow for collection and analysis of larger genetic sample sizes (Solberg et al. 2006; Stenglein et al. 2010).

Non-invasive DNA analyses have been successfully used to study the genetics of a wide variety of animals (Table 1), including for instance amphibians (Pidancier et al. 2003; Broquet et al. 2007), birds (Kormann et al. 2012; Olah et al. 2016), carnivores (Scandura et al. 2006; Stenglein et al. 2010; Rodgers and Janecka 2013), molluscs (Armbruster et al. 2005; Regnier et al. 2011; Morinha et al. 2014) and rodents (Pelz et al. 2007; Hamann et al. 2010; Reiners et al. 2011).

The multitude of options for non-invasive genetic sampling in virtually any species and the ethical concerns surrounding animal research raise the question: are the available non-invasive techniques being used?

Methods

To provide an overview of the current implementation status of non-invasive versus lethal and invasive DNA sampling, I conducted a systematic literature search (Pullin and Stewart 2006) for most recent wildlife genetics studies on either amphibians, birds, carnivores, molluscs, or rodents using the ISI Web of Science database in March 2019. These animal categories were chosen to represent different types of animals, for which non-invasive techniques were successfully implemented in the past (Table 1). The search term used was "genetic*", and "amphibian", "bird", "carnivore", "mollusc", or "rodent" were each included separately using the AND operator. The search was limited to studies published in 2017 and 2018. I then delimited the search results to relevant study areas by using the 'refine' function to include only studies in the fields of zoology, ecology, biodiversity conservation, genetics and heredity, evolutionary biology, and biology. Articles retrieved by the search were screened for their relevance to the topic. I followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009) for reporting the search results (Fig. 1). Specifically, after searching (stage "Identification" in Fig. 1), I used the title and abstract to select studies in which the animal category was the main object of genetic research (stage "Screening" in Fig. 1). For instance, amphibian studies that focused solely on chytrid infection were excluded. I then read the full text of the study to extract the information for this article, excluding studies that a) were not using genetics, b) used captive animals, c) used museum samples, already dead animals or samples from previous studies, d) were reviews, e) were only methods or model descriptions, or f) in which the method used was unclear (stage "Eligibility" in Fig. 1). Remaining relevant studies (stage "Included" in Fig. 1) were classified as using either 1) a lethal method if animals were killed before their DNA was sampled, 2) an invasive method if blood or tissue were sources of DNA, or 3) a non-invasive method if DNA sampling did not affect the physical integrity of the animal (Lefort et al. 2019).

Results

In total, I identified 181 relevant studies, covering wide range of species (Fig. 1; Suppl. material 1: Table S1). The studies on amphibians included frogs, toads, salamanders and newts, carnivores encompassed genets, leopards, wolves, cheetahs, bears, martens, lynxes, fishers, foxes, coyotes, Tasmanian devils, minks, bobcats, racoons, tigers, skunks, jaguars and wild cats, molluscs included marine snails, nudibranchs, mussels and other marine molluscs, and rodents included mice, voles, dormice, mole-rats, rats, hamsters, deer mice, tuco-tucos and gerbils. The rather generic search term "genetic*" captured different types of studies in wildlife genetics utilizing a variety of genetic markers (Fig. 2). Lethal, invasive, non-invasive and both invasive and non-invasive DNA sampling methods were all used in each type of study. Exceptions were genome-wide association studies, studies on hybridization and phylogenetics studies, which used only lethal and invasive sampling (Fig. 2), most likely due to the perceived necessity for high-quality DNA (Carroll et al. 2018).

In amphibians, the most common method used was lethal sampling (34%), followed by toe clipping (32%) and tail clipping (18%). Buccal swabs and skin swabs were used for DNA collection in 12% and 2% of the studies, respectively. Two percent of studies on amphibians used both invasive and non-invasive methods (Fig. 3). Blood sampling was the most common method for obtaining DNA in birds (76%), followed by studies using both invasive and non-invasive methods (10%) and lethal sampling (6%). A non-invasive method, in this case the use of feathers, was utilized in 8% of the reviewed studies (Fig. 3). Carnivore studies had the highest proportion of non-invasively obtained samples: 58% of studies used faeces, 23% used hair for DNA extraction. Ear clipping (6%) and blood sampling (6%) were also implemented, and 6% of studies used both invasive and non-invasive methods (Fig. 3). A lethal method of DNA sampling was implemented in all the reviewed studies on molluscs (Fig. 3). In rodents, lethal sampling was used in approximately half of the reviewed studies (56%), followed by

Table 1. Examples of studies using non-invasive DNA sampling in the selected animal categories.

Category	DNA source	Reference
Amphibian	Buccal swabs	Broquet et al. 2007; Angelone and Holderegger 2009
	eDNA	Goldberg et al. 2011; Olson et al. 2012
	Skin swabs	Prunier et al. 2012; Pichlmuller et al. 2013
Bird	Buccal swabs	Handel et al. 2006; Adam et al. 2014
	Eggshells	Egloff et al. 2009; Kjelland and Kraemer 2012
	Faeces	Idaghdour et al. 2003; Rosner et al. 2014
	Feathers	Segelbacher 2002; Rudnick et al. 2007
Carnivore	Faeces	Chaves et al. 2012; Sollmann et al. 2013
	Hair	Castro-Arellano et al. 2008; Ausband et al. 2011
	Saliva	Harms et al. 2015; Lobo et al. 2015
Mollusc	eDNA	Ardura et al. 2015; Cho et al. 2016
	Mucus	Armbruster et al. 2005; Palmer et al. 2008
Rodent	Buccal swabs	Parmenter et al. 1998; Mitrecic et al. 2008
	Faeces	Alasaad et al. 2011; Mestre et al. 2015
	Hair	Reiners et al. 2011; Barja et al. 2016

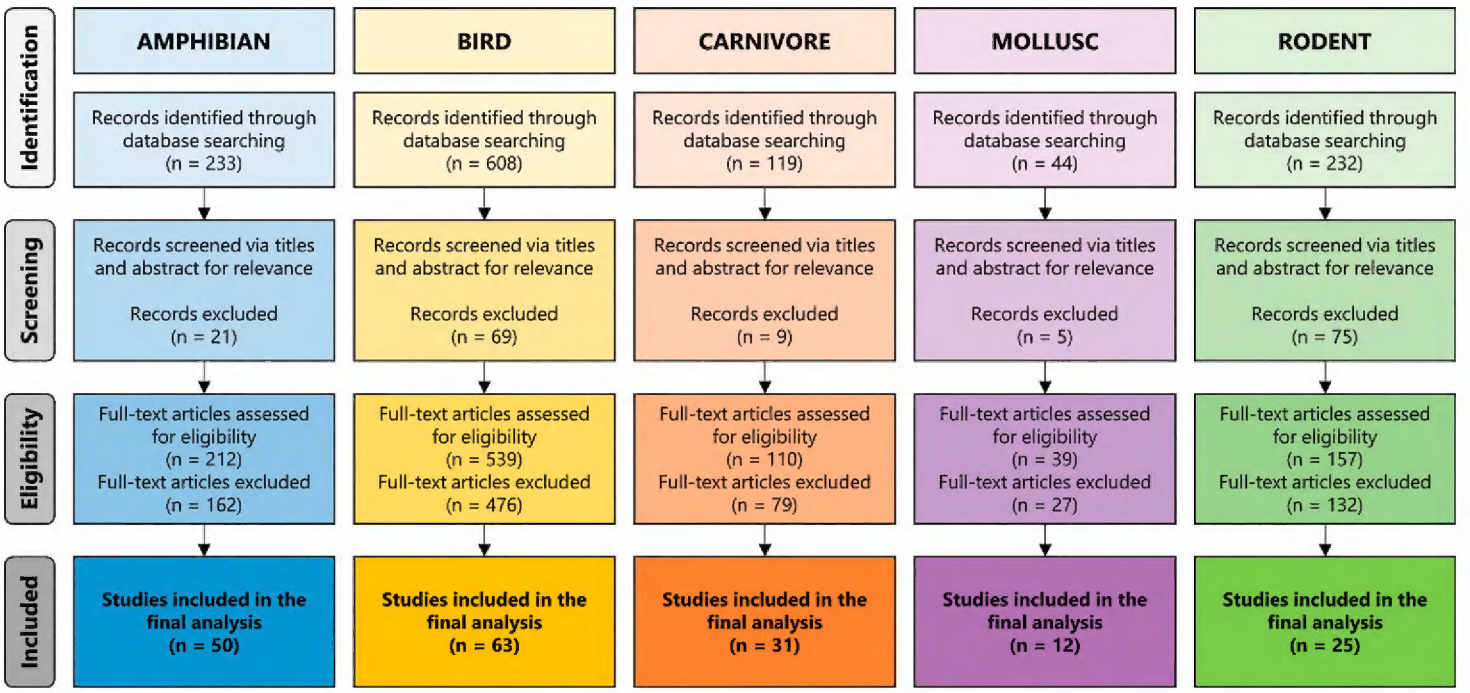
Identification
Screening
Eligibility
Included
AMPHIBIAN
BIRD
CARNIVORE
MOLLUSC
RODENT
Records identified through database searching (n = 233)
Records identified through database searching (n = 608)
Records identified through database searching (n = 119)
Records identified through database searching (n = 44)
Records identified through database searching (n = 232)
Records screened via titles and abstract for relevance
Records excluded (n = 21)
Records screened via titles and abstract for relevance
Records excluded (n = 69)
Records screened via titles and abstract for relevance
Records excluded (n = 9)
Records screened via titles and abstract for relevance
Records excluded (n = 5)
Records screened via titles and abstract for relevance
Records excluded (n = 75)
Full-text articles assessed for eligibility (n = 212)
Full-text articles excluded (n = 162)
Full-text articles assessed for eligibility (n = 539)
Full-text articles excluded (n = 476)
Full-text articles assessed for eligibility (n = 110)
Full-text articles excluded (n = 79)
Full-text articles assessed for eligibility (n = 39)
Full-text articles excluded (n = 27)
Full-text articles assessed for eligibility (n = 157)
Full-text articles excluded (n = 132)
Studies included in the final analysis (n = 50)
Studies included in the final analysis (n = 63)
Studies included in the final analysis (n = 31)
Studies included in the final analysis (n = 12)
Studies included in the final analysis (n = 25)

Figure 1. PRISMA flow diagram identifying the number of records excluded at each stage of the literature review process.

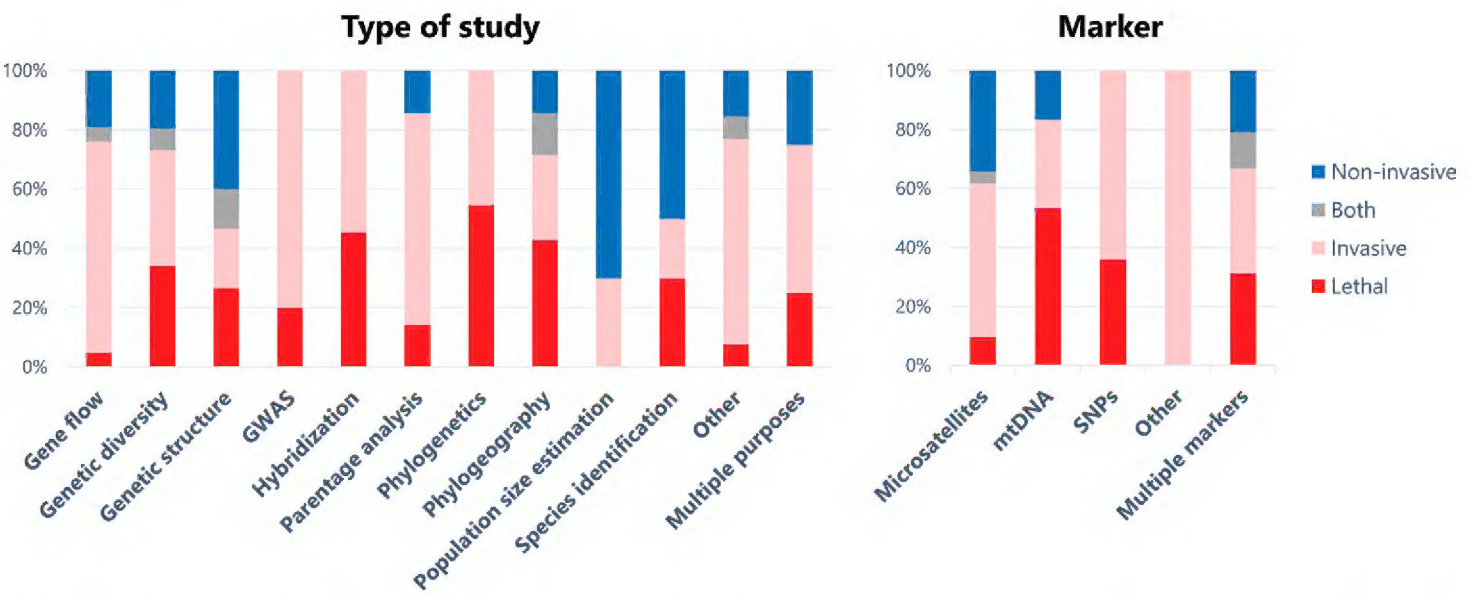

Figure 2. Proportion of DNA sampling techniques (lethal, invasive, non-invasive, or both invasive and non-invasive) used in the identified relevant genetics studies, categorized by the research objective of the study (Type of study; left) and the genetic marker (Marker; right).

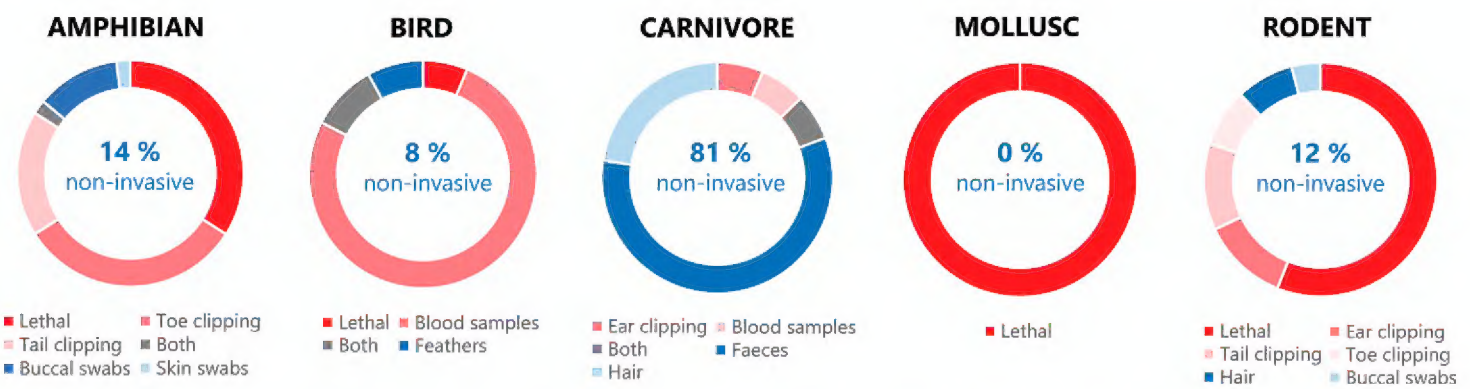

Figure 3. Proportion of identified relevant studies on genetics in amphibians, birds, carnivores, molluscs and rodents published in 2017–2018. Lethal and invasive methods of DNA collection are in red tones, non-invasive in blue tones, grey symbolizes that both invasive and non-invasive methods were used for DNA collection. Except for studies on carnivores, majority of studies used a lethal or an invasive genetic sampling technique.

ear clipping (12%), tail clipping (12%) and toe clipping (8%). Non-invasive methods, DNA extraction from hair (8%) and buccal swabs (4%), constituted a minority (Fig. 3).

In majority of the studies, the choice of a sampling method was not justified. The reason was mentioned in only 15 studies (out of 181; Suppl. material 1: Table S1), either implicitly (e.g. non-invasive method used for an endangered species; lethal sampling of animals that were collected also as museum vouchers) or explicitly (e.g. toe clipping used also as a marking technique, blood or skin swabs also used for parasite detection, tail clipping in salamanders used because of their ability to regenerate lost limbs).

Discussion

Here, I presented a snapshot of the latest implementation status of non-invasive genetic sampling in wildlife genetics studies. Only 22% of the studies identified as relevant conducted DNA sampling through a non-invasive method. This is despite the fact that suitable non-invasive alternatives are already available (Table 1). Even though my analysis may have not captured all published studies, the results clearly indicate that ecologists and wildlife biologists seem to struggle to implement non-invasive genetic sampling into their research.

The highest proportion of non-invasive methods was in studies on carnivores. This is not surprising, as carnivores are often elusive and potentially dangerous animals and capturing them is more difficult than catching molluscs for instance. Moreover, there are often stringent criteria imposed on biologists to attain research permits for obtaining samples from mammals (Sikes and Bryan 2015). In contrast, most invertebrates are not protected by the current animal welfare laws and research on them does not require assessment and approval by ethics committees (Directive 2010/63/EU). In this way, scientists may not be compelled to consider more animal welfare-friendly alternatives in all species. The requirement to conduct proper literature searches on non-invasive methods before commencing research in both vertebrate and invertebrate animals thus needs to be strictly enforced. For example, researchers should demonstrate to the ethics committees that they are aware of the existing non-invasive methods, and that these have been at least considered – if not implemented.

Another obstacle that might be preventing the wider uptake of non-invasive methods is limited awareness of alternatives and more ethical approaches to research due to lack of education and discussion about animal ethics and animal welfare in ecological research (Bekoff 2002; Zemanova 2017). For some scientists it may be also difficult to abandon methods that they either developed, were taught or have taught to their students (Gruber and Dewhurst 2004). The psychological factors should not be underestimated (Knight 2011). Better support for raising awareness and implementation of non-invasive techniques is therefore critical. This could be done for instance by introducing compulsory classes on animal welfare to all undergraduate students in biology, as has been done in some veterinary schools worldwide (Hewson et al. 2005; Mota-Rojas et al. 2018).

Lastly, the development of new and implementation of existing non-invasive methods can be costly, until trial and error leads to establishment of an efficient protocol. And yet, opportunitiesv to conduct research on developing non-invasive techniques tend to be part of larger funding calls that focus on other topics (Prescott et al. 2017). Strategic grant calls for the development non-invasive methods within national as well as global funding schemes could help the scientific community to meet the research objectives while addressing societal concerns for animal well-being.

Conclusions

This review should serve as an appeal to 1) ecologists and wildlife biologists to inform themselves about and implement existing non-invasive genetic sampling techniques whenever possible, 2) ethics committees to require the use of the least harmful research method, and 3) funding bodies to support and promote this endeavour. Change may not always be convenient, but it is necessary for ensuring welfare of research animals as well as public support of science.

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Author contribution

Miriam A. Zemanova developed, designed, and wrote the manuscript.

Authors	Contribution	ACI
MAZ	1.00	NA

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Supplementary material 1

Table S1. Peer-reviewed studies on wildlife genetics in amphibians, birds, carnivores, molluscs and rodents, published in 2017–2018, which were included in the review

Authors: Miriam A. Zemanova

Data type: reference data

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